EVALUATION OF *Rhyzobius lophanthae* (BLAISDELL) AND *Cryptolaemus montrouzieri* MULSANT (COLEOPTERA: COCCINELLIDAE) AS PREDATORS OF *Aulacaspis yasumatsui* TAKAGI (HEMIPTERA: DIASPIDIDAE)

By

GRETA THORSON

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To my family for their constant support and encouragement, as well as past and present colleagues and mentors who helped inspire me along the way
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The objective of this research was to gain a better understanding of *Rhyzobius lophanthae* (Blaisdell) and *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) as augmentative biological control agents of *Aulacaspis yasumatsui* Takagi (Hemiptera: Diaspididae), a severe pest on *Cycas revoluta* Thunberg. This study quantitatively evaluated the consumption of *A. yasumatsui* by both predators at 18 and 24° C. The larval developmental periods, pupation periods, adult longevities, and fecundities of the two predators were compared. The effect of releases of *R. lophanthae* on *A. yasumatsui* populations and the time frame in which control can be seen on an infested plant were examined through a greenhouse and field study.

Both *R. lophanthae* and *C. montrouzieri* were able to complete larval development when feeding on male and female *A. yasumatsui* whereas only *R. lophanthae* completed larval development when feeding only on female scales at both temperatures. Larval survivorship was significantly greater in *R. lophanthae* than in *C. montrouzieri*. Larval development time and pupation period were significantly greater for *C. montrouzieri* when feeding on both male and female scales. Female *R. lophanthae* are able to produce eggs when feeding on *A. yasumatsui*, whereas *C. montrouzieri* are unable to produce eggs at 18°C or 24°C. This study revealed the rates of consumption by *R. lophanthae* were higher than that of *C. montrouzieri* as well as the
ineffectiveness of both beetles in targeting female *A. yasumatsui*, implicating their inability to control the increase of scale populations.

In the greenhouse study, treatments with higher numbers of beetles consumed the greatest mean number of scales and a greater proportion of the total scale population. Healthy scale infestations were reduced by 10% following the introduction of *R. lophanthae* in treatments with 4 and 6 beetles whereas treatments with 2 beetles were reduced by 35%. Initial beetle feeding damage was observed on infested plants during the first 8 d during a field study where plants were treated with 0, 100, 200, or 300 beetles. With the absence of beetles and low numbers of larvae, there were no significant differences in damage to scales among treatments at later time points. The level of scale infestation increased over the course of the study following release of *R. lophanthae* indicating the ineffectiveness of the predator.
CHAPTER 1
REVIEW OF LITERATURE

Introduction

*Cycas revoluta* Thunberg, commonly referred to as king sago, is a popular plant in the landscape environment of Florida, being widely distributed throughout the state in botanical gardens and residences. Since 1996, *C. revoluta* in Florida has been attacked by the cycad aulacaspis scale, *Aulacaspis yasumatsui* Takagi. This scale not only presents a substantial threat to landscape cycad plants in Florida, but also worldwide. The scale is native to a region stretching from the Andaman Islands to Vietnam, Thailand, southern China, and likely Cambodia, Laos, Malaysia, and Myanmar (Howard et al. 1999; Muniappan 2005). The first known outbreak outside of its native range occurred at the Bogor Botanical Garden in Java in the 1980s (Haynes 2005). Since its introduction in the US, *A. yasumatsui* has spread to Alabama, California, Georgia, Hawaii, Louisiana, South Carolina, and Texas (Broome 2000). Its destruction is also seen in the West Indies, Guam, Hong Kong, Singapore, Taiwan, New Zealand, Costa Rica, and Africa (Weissling et al. 1999; Hodges et al. 2004; Moore et al. 2005; Germain and Hodges 2007). Threats of further spread are a major concern to Australia and India which currently do not have any recorded outbreaks (Muniappan and Viraktamath 2006).

The scale destroys plants and gives them an unsightly snow-covered appearance. Heavy infestations form a dense multilayered covering of nearly 465 scales per square centimeter (Weissling et al. 1999) and result in the death of the cycad (Heu 2003). The scale is successful in Florida due to the warm temperatures which allow development from egg to adult in less than one month; females produce 100 eggs on average. Initial infestations in Miami were treated with systemic insecticides from October 1996 to January 1998, although re-infestations were common (Howard et al. 1999).
**Aulacaspis yasumatsui Takagi**

*Aulacaspis yasumatsui* was originally described from Bangkok, Thailand in 1972 (Takagi 1977). Belonging to the family Diaspididae, these scales have a waxy outer coating that forms a protective barrier for both adult and eggs (Weissling et al. 1999). Females (Figure 1-1) are 1.2-1.6mm in length and pyriform in shape with an irregular pear-shaped covering, whereas males (Figure 1-2) are 0.5-0.6 mm in length, with tricarinate coverings. Males are much more abundant on infested plants than females (Howard et al. 1999).

In 1996, *A. yasumatsui* was found infesting ornamental cycad plants in Miami, Florida. The pest quickly spread throughout southern Florida, aided by the ornamental industry’s transport of cycad plants (McLaughlin 1998). Howard et al. (1999) observed that within 16 days from initial infestation during April and June, a mean infestation of 69.6 scales per leaflet, with 84.8% being in first instar and the remainder in second instar, could be seen on the abaxial surface of *C. revoluta* leaflets. Approximately 18.1% of scales were in the third instar after 28 days and 89.1% after 41 days, 68.2% of which had produced eggs. Number of days for development was significantly less between August and September, but resulted in similar scale densities.

Studies have shown that *Cycas* species native to China often had significantly higher infestations of *A. yasumatsui* than other host plants (Howard et al. 1999). This suggests that cycads in China were likely the original host plants of scales because *A. yasumatsui* readily uses *Cycas* species as hosts, while infestations on cycad species in other genera are considerably less frequent. *Aulacaspis yasumatsui* has been found infesting cycads of the families Cycadaceae, Zamiaceae, and Stangeriaceae (Howard et al. 1999).

*Aulacaspis yasumatsui* causes injury by piercing plant tissue with its stylet mouthparts and sucking sap out of the leaves. As a result, necrosis of the leaves is evident by the yellow-brown
coloration beginning at the leaflet tips. Initial infestations are seen on the underside of leaves and, as the population increases, scales move to the topside of leaflets. A heavy buildup of scales is often observed by a snowy appearance detectable up to 2 m away.

Control of *A. yasumatsui* is difficult due to several factors. First, it was discovered that the scale is capable of infesting the primary and secondary roots of plants as deep as 60 cm into the ground, where they are not easily observed, and can therefore be transported unknowingly (Howard et al. 1999). Additionally, scales can escape freezing temperatures by hiding in overlapping plant material from previous years’ growth in the central cone of *C. revoluta* plants which cannot be reached with normal chemical treatments (Broome 2002). Immediate control was further hindered by the strong resemblance of *Pseudaulacaspis cockerelli* (Comstock) (Figure 1-3) and *Pinnaspis strachani* (Cooley) to *A. yasumatsui* which can be differentiated by its orange-colored body and eggs and swollen prostigma. Initial control measures targeting *P. cockerelli* were ineffective against this pest which was later correctly identified by Dr. Avas B. Hamon, Department of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville (Howard et al. 1996).

Chemical control of *A. yasumatsui* has evolved quickly in the last ten years in an effort to control this invasive scale from moving to other parts of the U.S. The earliest treatment recommended drenching plants with malathion (Walters et al. 1997; Weissling et al. 1999). Malathion is a fast acting, broad spectrum insecticide that can be toxic if misused, causing phytotoxicity in new growth and death of beneficial organisms (Hodges et al. 2003; Emshousen and Mannion 2004b).

Horticultural oil and fish oil were also among the first chemicals used to control the spread of *A. yasumatsui*. Organocide (95% fish oil) consistently reduced scale populations when
infestations were mild. Treatment with horticultural oil has also been effective, but full coverage of infested plants is difficult primarily due to plant architecture wherein cycad leaves curl down and inward. Oil sprays were unable to be applied in uniform thickness and result in a build up of past oil treatments after several applications (Hodges et al. 2003). Weekly or biweekly sprayings for several months is required to control scales on leaflets and stems (Walters et al. 1997; Meyerdirk 2002; Hodges et al. 2003). Treatment with Organocide and Ortho Horticultural Mineral Oil in studies by Caldwell (2003) resulted in less then 50% control on the undersides of leaves where *A. yasumatsui* are most dense. Mixed treatments with oil and the contact insecticide Sevin (carbaryl), resulted in greater scale mortality than oil treatments alone, but increase the potential to negatively affect biological control agents (Hodges et al. 2003).

Efforts to control scales on all parts of infested plants including the roots have included application of a soil drench containing up to 0.1 L of imidacloprid per 18.9 L of water; however, the label rate proved to be not as effective in controlling heavy scale infestations (Howard and Weissling 1999; Hodges et al. 2003; Emshousen and Mannion 2004b).

Cygon, with the active ingredient dimethoate, has been applied to plants in studies with overwintering nymphs from mid-May, June, and July by drenching the entire plant (Caldwell 2003). Root-infesting *A. yasumatsui* were treated by soil drenching with Cygon 2E at a rate of 0.06 L per 3.8 L of water. Cygon 2E caused mortality in 85% of crawlers on the underside of leaves. Root drench treatments resulted in 95% mortality of scales 31 d after treatment. This percentage was significantly higher than natural mortality of 16% in the control. Application of this chemical has proven to be highly detrimental to natural enemies. Despite its effectiveness, Cygon 2E is not labeled for use on cycads and is thus illegal to use on infested plants. The EPA
published in the Federal Register a cancelation notice of dimethoate products, including Cygon, for residential use (U.S. EPA 2002).

A study by Emshousen and Mannion (2004a) revealed that Distance, with the active ingredient pyriproxyfen, an insect growth regulator, provided excellent control of A. yasumatsui. Distance acts to inhibit metamorphosis, therefore making it less likely to affect biological control agents and humans. This insecticide’s effectiveness in disrupting the development of scales requires a whole life cycle in order to provide control. Treatments with Distance have resulted in 100% mortality of eggs and 99% of adult females after 8 weeks. Due to threats of A. yasumatsui moving to other cycad species in Florida, pyriproxyfen was tested on many of these plants. Treatments were able to control at least 75% of adult female A. yasumatsui and eggs on lightly infested plants. Distance did not control scale well on densely infested plants, but showed no signs of phytotoxicity.

It is difficult to eliminate A. yasumatsui because scales can be transported unknowingly on the roots of plants and unsettled crawlers can become airborne up to a half mile (Broome 2000). Due to scale outbreaks and the inability to control them in Florida, there are severe restrictions on the export of C. revoluta outside of Florida. Mortality of 70-100% of the ornamental cycads in Hong Kong has been attributed to this same pest, and may be indicative of the level of destruction possible in Florida (Hodgson and Martin 2001). This suggests that there is a need for better understanding the influence of biological control agents on scale control and the development of methods for effective release of these agents.

Two natural enemies, the parasitoid Coccobius fulvus (Compere and Annecke) and the predator Cybocephalus nipponicus Endrody-Younga, were released in Florida in 1998 to control scales (Howard et al. 1999; Hodges et al. 2003; Muniappan 2005). Re-releases showed that
control is possible during some parts of the year (Hodges et al. 2003). Further studies of *C. fulvus* by Wiese and Mannion (2005) indicate that the wasp was successfully parasitizing scales, although the beetles have failed to significantly control the scale.

Sixteen lady beetles (Coccinellidae) have been identified on cycads infested by *A. yasumatsui* in southern Florida (Cave 2006). *Rhyzobius lophanthae* (Blaisdell), an Australian lady beetle, has been observed feeding on *A. yasumatsui*, but isolated populations have been found only in downtown Tampa and Tallahassee (Cave 2006). *Rhyzobius lophanthae* has been an effective biological control agent in Hawaii, Italy, and Guam, but its limited distribution in Florida could be hampering its effectiveness as a biological control agent in terms of dispersal across all of southern Florida. Another Australian lady beetle, *Cryptolaemus montrouzieri* Mulsant, has been frequently encountered throughout southern Florida on *C. revoluta* plants infested with *A. yasumatsui*. Research into the effectiveness of both species of lady beetles as predators of *A. yasumatsui* would be useful in determining their significance as biological control agents.

*Rhyzobius lophanthae* (Blaisdell)

*Rhyzobius lophanthae* (= *Lindorus lophanthae* Blaisdell) has been identified as an important natural enemy of many armored scale species (Yus 1973; Rosen 1990). The beetle was introduced from New South Wales into California by Albert Koebele between 1889 and 1892, and successfully controlled black scale, *Saissetia oleae* (Bern) (Greathead 1973). In 1894, *R. lophanthae* was introduced into Hawaii to control *Aspidiotus destructor* Signoret (Honda 1995; McLaughlin 1998). Subsequently, *R. lophanthae* has been recommended for release in many insect pest management programs. *Rhyzobius lophanthae* has proven to be an effective biological control agent worldwide, specifically controlling *Carulaspis juniperi* (Bouché) in Italy, *Chrysomphalus dictyospermi* (Morgan) in Morocco, *Parlatoria blanchardi* (Targioni) in Israel,
Aulacaspis tegalensis (Zhut) in East Africa, and Aspidiotus nerii Bouché in Greece. However, *R. lophanthae* failed to control *Aonidiella aurantii* (Maskell) in California (Honda 1995). Although *R. lophanthae* currently lives in areas of Florida where *A. yasumatsui* is a problem, the beetle has been unable to regulate pest populations through natural interactions, leading to concerns over the ability of *R. lophanthae* to disperse to isolated plants in the urban and suburban landscapes.

*Rhyzobius lophanthae* (Figure 1-4) is a small predatory beetle, with adult females approximately 2.5 mm in length and 1.8 mm in width. Adult males are 2.4 mm in length and 1.7 mm in width. The head in both sexes is reddish-brown in color and covered with setae. The antennae are nine-segmented and the elytra are black-brown (Smirnoff 1950; Stathus 2002). There are currently no data quantifying the capacity of *R. lophanthae* to prey upon *A. yasumatsui*. Consumption on other prey and fecundity was measured by Stathus (2000) in Greece.

There is concern about the adequate number of *R. lophanthae* needed to control an infestation of *A. yasumatsui* on a plant. Two companies, Gardening Zone and IPM of Alaska, both suggest this predator should be augmentatively released onto cycads to facilitate control of *A. yasumatsui*. These companies recommend releasing 20-40 beetles for each infested plant, but are unclear about the plant size treated.

**Cryptolaemus montrouzieri** Mulsant

*Cryptolaemus montrouzieri* (Figure 1-5) was imported from Australia into California in 1872. It is used frequently in the biological control of mealybugs and soft scales (Cooper 1985; Heidari and Copland 1992) Adults range between 3.4 and 4.5 mm in length and between 2.4 and 3.1 mm in width. The head, prothorax and elytral apices are reddish in color; the remainder of the body is black. Its dorsal surface is densely punctate, apart from the humeral callus which is almost devoid of punctures. Apart from being found in California, *C. montrouzieri* is also
distributed throughout central and southern Florida (Gordon 1985). In feeding studies comparing
*C. montrouzieri* to another predatory beetle, *Adalia bipunctata* (Linnaeus), it was discovered that
both beetles are not selective feeders and that, overall, *C. montrouzieri* eats six times fewer
scales and has a smaller gut capacity (Magro et al. 2002). Fecundity life tables were developed
based on studies of *C. montrouzieri* feeding on the pink hibiscus mealybug, *Maconellicoccus
hirsutus* (Green), in Trinidad (Persad et al. 2002). Because *C. montrouzieri* is frequently
encountered on cycads infested with *A. yasumatsui* (Cave 2006), a comparative feeding study
would indicate the effectiveness of *C. montrouzieri* as a biological control agent of the pest.

**Objectives**

The aim of this study was to determine how the life history and feeding behavior of *R.
lophanthae* and *C. montrouzieri* compare when *A. yasumatsui* on *C. revoluta* is provided as prey.
The research also studied the effectiveness of *R. lophanthae* in field release trials. Therefore,
data were collected to answer these specific questions:

- Are the consumption rates of *R. lophanthae* and *C. montrouzieri* larvae and adults feeding
  on *A. yasumatsui* significantly different at two different temperatures?

- Are the larval development times, pupation periods, adult longevities, and female
  fecundities of *R. lophanthae* and *C. montrouzieri* preying on *A. yasumatsui* significantly
  different?

- How many *R. lophanthae* adults should be released on a plant to provide significant
  control of *A. yasumatsui* and how long do the beetles persist on the plant?
Figure 1-1. Adult female Aulacaspis yasumatsui (Hodges et al. 2003).
Figure 1-2. Adult male Aulacaspis yasumatsui.
Figure 1-3. Adult female Aulacaspis yasumatsui and Pseudaulacaspis cockerelli (Hodges et al. 2003).
Figure 1-4. Dorsal and ventral view of adult and 3\textsuperscript{rd} instar *Rhyzobius lophanthae* (Cave 2006).
Figure 1-5. Dorsal and ventral view of adult and 3rd instar *Cryptolaemus montrouzieri*.
CHAPTER 2
LIFE HISTORY OF RHYZOBIIUS LOPHANTHAE AND CRYPTOLAEMUS MONTROUZIERI
FEEDING ON AULACASPIAS YASUMATSUI

Introduction

Members of the family Coccinellidae, commonly called lady beetles, are the most widely used predator in biological control programs and are successful in controlling aphids, mealybugs, scales, whiteflies, psyllids, and mites (Obrycki and Kring 1998). Since the 1890s, nearly 40 coccinellid species have been introduced into the U.S. for control of pest insects (Frank and McCoy 2007).

*Rhyzobius lophanthae*, a species of lady beetle native to New South Wales, was first introduced into California for control of black scale, *Saissetia oleae* (Olivier) (Greathead 1973). This lady beetle has been effective in the biological control of *Aspidiotus destructor* Signoret in Hawaii, *Carulaspis juniperi* (Bouché) in Italy, *Chrysomphalus dictyospermi* (Morgan) in Morocco, *Parlatoria blanchardi* (Targioni) in Israel, *Aulacaspis tegalensis* (Zhut) in East Africa, and *Aspidiotus nerii* Bouché in Greece (Honda and Luck 1995; McLaughlin 1998). The short developmental period, lack of parasitoids, absence of diapause, and consumption of pest populations by both larvae and adults makes *R. lophanthae* successful in biological control programs (Stathas 2000).

*Cryptolaemus montrouzieri*, known commonly as the mealybug destroyer, is a generalist feeder native to Australia and has been used primarily to control *Planococcus citri* (Risso), a pest in citrus. Introductions have also been made to control *Coccus viridis* (Green), *Pulvinaria psidii* (Maskell), *Dysmicoccus bonensis* (Kuwana), *Dysmicoccus brevipes* (Cockerell), *Ferrisia virgata* (Cockerell), *Maconellicoccus hirsutus* (Green), *Nipaecoccus nipae* (Maskell), *Planococcus citri* (Risso), *Pseudococcus comstocki* (Kuwana), *Pseudococcus longispinus* (Targioni-Tozzetti), *Pseudococcus maritimus* (Ehrhorn), *Pseudococcus viburni* (Signoret), *Saccharicoccus sacchari*
(Cockerell), *Dactylopius tomentosus* (Lamarck), and *Eriococcus araucariae* (Muskell) (Frank and McCoy 2007). The lady beetle is distributed throughout central and southern Florida (Gordon 1985).

Considerable loss of cycads in the urban landscape which has been attributed to the cycad aulacaspis scale, *Aulacaspis yasumatsui*, has resulted in decreased production of cycads by the ornamental industry in Florida (Hodges et al. 2003). The scale was introduced accidentally into the U.S. from Thailand (McLaughlin 1998), with the initial detection occurring in Miami, FL in 1998. *Aulacaspis yasumatsui* causes injury by piercing plant tissue to remove plant sap. As a result, necrosis of the leaves is evident by the yellow-brown coloration beginning at the leaflet tips and leads to eventual death of plants. Initial infestations are seen on the underside of leaves and, as the population increases, scales move to the topside of leaflets. *Aulacaspis yasumatsui* has been found infesting plant species in the families Cycadaceae, Zamiaceae, and Stangeriaceae (Howard et al. 1999).

Sixteen lady beetle species have been identified on cycads infested by *A. yasumatsui* in southern Florida (Cave 2006). Of these, *R. lophanthae* and *C. montrouzieri* were selected for this study based on their success in other biological control programs. There is no knowledge of the development time, longevity, and fecundity of *R. lophanthae* and *C. montrouzieri* feeding on *A. yasumatsui* on *Cycas revoluta* plants. Therefore, this study was conducted to obtain this information in order to compare the efficacy of both lady beetles as biological control agents of *A. yasumatsui* in Florida.

**Materials and Methods**

**Insects**

*Aulacaspis yasumatsui* was reared on *C. revoluta* plants in 3.7 L pots and maintained in a greenhouse with 30% RH. Plants were fertilized and watered regularly according to grower’s
recommendations to maintain the health of plants. Uninfested *C. revoluta* were exposed to infested plants with active crawlers by interlocking leaflets for 1 week, allowing crawlers to settle on the clean foliage. Moderately infested plants with 2nd and 3rd instar *A. yasumatsui* were obtained in approximately 1 month following exposure at 30°C. Adult *R. lophanthae* were obtained from Rincon-Vitova Insectaries (Ventura, California) and kept in 20 × 20 × 20 cm Bug Dorms (BioQuip, Inc., Rancho Dominguez, CA) with water-saturated cotton balls and infested *C. revoluta* plants at 25°C, 60% RH and 14:10 (L:D) photoperiod. Eggs of *R. lophanthae* used in this study were produced from females feeding on *A. yasumatsui*. Adult *C. montrouzieri* were unable to produce eggs while feeding on *A. yasumatsui* (personal observation), therefore, eggs were obtained from the Florida Department of Agriculture and Consumer Services, Division of Plant Industry in Gainesville, FL. Eggs of *C. montrouzieri* were produced from females feeding on *M. hirsutus*.

**Experimental Design**

Eggs and pupae of *R. lophanthae* and *C. montrouzieri* were placed individually in 20 × 9 mm Petri dishes with screen lids (Figure 2-1). Following larval and adult emergence, the predators were supplied daily with fresh *C. revoluta* leaflets infested with 10-20 2nd and 3rd instars of male and female or just female *A. yasumatsui*. Ten larvae and ten adults of each sex of both predator species were kept in environmental chambers set at 18±2°C and 24±2°C with 60% relative humidity and 14:10 (L:D) photoperiod. Predators were checked daily for molting and death. Adult longevity and age specific survivorship were measured in this study.

Ten mating pairs of *R. lophanthae* and *C. montrouzieri* were isolated for 48 h in 20 × 9 mm Petri dishes with screen lids and lined with moist filter paper. Pairs were selected based on observation of copulation. Dishes were supplied daily with *C. revoluta* leaflets infested with ~20 2nd and 3rd instars of male and female *A. yasumatsui*. Adult females were then placed
individually into Petri dishes for the remainder of their life and provided new *C. revoluta* leaflets with scales *ad libitum*. Presence of predator eggs was checked by visually inspecting leaflets and manually removing the cover of female scales. The number of eggs produced and number of females alive were recorded daily to determine fecundity.

Each study (set of 10 females) was repeated five times for both temperatures. Means of development time, pupation period, longevity, and fecundity were statistically compared between predator species and temperatures using an analysis of variance (ANOVA) (Proc GLM, SAS Institute, 2001) and a t-test to separate means. Data for all 5 studies were combined during the analysis. Means are reported with their standard error. Population growth parameters of *R. lophanthae* feeding on female *A. yasumatsui* at both temperatures were calculated by computation of the net reproductive rate (*R*₀ = 1ₓ mₓ), the intrinsic rate of increase (*r*ₘ = (ln *R*₀) / *T*), and mean generation time (*T* = ∑ 1ₓ mₓ *x* / ∑ 1ₓ mₓ).

**Results**

**Egg Duration**

Duration time for *R. lophanthae* eggs from females reared on *A. yasumatsui* was significantly greater (t=8.9693, df=97, P<0.001) than that for *C. montrouzieri* eggs reared on *Maconellicoccus hirsutus* at 18°C (Figure 2-2). There was no significant difference (t=0.4471, df=97, P>0.5) between species at 24°C.

**Female Scales Only As Prey for Larvae**

Survivorship of larvae feeding only on female scales varied between the two predators and the two temperatures (Figure 2-3). There was a sharp decrease in survivorship at 18°C within the first 20 days for *R. lophanthae* larvae and likewise was observed until day 18 for *C. montrouzieri*. At 24°C, larvae of both species had a sharp decrease in survivorship within the first 7 days. Cohort survivorship was 50% on day 10 for *R. lophanthae* and on day 12 for *C.*
montrouzieri at 18°C, whereas 50% survivorship of the cohort at 24°C occurred on day 4 for R. lophanthae and on day 7 for C. montrouzieri.

At 18°C, 2nd instar R. lophanthae developed in the fewest number of days (6.8 ± 0.4), whereas the longest development time was observed during the 4th instar (17.1 ± 1.4 d) (Figure 2-4). Development time in the 4th instar was almost twice as long as all other instars, with 1st and 3rd instars developing in 10.3 ± 0.6 d and 9.7 ± 0.6 d, respectively. Development time was not significantly different between 1st and 3rd instars. Total larval development time of R. lophanthae averaged 43.9 ± 2.9 d at 18°C. The minimum development time was 38 d and the maximum was 64 d. At this temperature, larvae of C. montrouzieri did not reach the 4th instar; larvae in the 3rd instar died in 1.0 ± 3.3 d. Larval development was longest during the 1st instar (5.8 ± 3.7 d) (Figure 2-4).

At 24°C, 3rd instar R. lophanthae developed in the fewest number of days (3.8 ± 0.3), whereas the longest development time was observed during the 4th instar (5.9 ± 0.3 d). Development time was identical (5.2 ± 0.3 d) for both 1st and 2nd instars (Figure 2-5). Total larval development time of R. lophanthae at 24°C averaged 20.1 ± 1.3 d, with a minimum of 17 d and a maximum of 23 d. Larvae of C. montrouzieri did not reach 2nd instar at 24°C. First instars died on average in 5.2 ± 2.6 d (n=36).

Comparisons of instar development time between species can only be made for 1st and 2nd instars at 18°C since no C. montrouzieri larvae survived the 3rd instar at 18°C and none survived the 1st instar at 24°C (Figures 2-4, 2-5). During the 1st (t= 5.3795, df= 50, P<0.0001) and 2nd (t= 8.6734, df= 23, P<0.0001) instars at 18°C, R. lophanthae took significantly longer to develop than C. montrouzieri.
Male and Female Scales as Prey For Larvae

Survivorship of *R. lophanthae* and *C. montrouzieri* larvae feeding on male and female *A. yasumatsui* decreased considerably within the first 25 days at 18°C and 24°C (Figure 2-6). Cohort survivorship was 50% on day 14 of development of *R. lophanthae* and on day 18 of *C. montrouzieri* at 18°C. Cohort survivorship at 24°C was 50% on day 12 of *R. lophanthae* and on day 14 of *C. montrouzieri*.

At 18°C, 2nd instar *R. lophanthae* developed in the fewest number of days (7.2 ± 1.3), whereas the longest development time was observed during the 4th instar (16.7 ± 4.2 d) (Figure 2-7). Development time in the 3rd and 4th instars was almost twice as long as development of 1st and 2nd instars. Total larval development time for *R. lophanthae* averaged 45.5 ± 20.1 d at 18°C, whereas *C. montrouzieri* total development time averaged 85.1 ± 15.0 d. Development time for *C. montrouzieri* was shortest during the 2nd instar (10.1 ± 2.9 d), whereas the longest development time was observed during the 4th instar (41.2 ± 7.3 d) at 18°C (Figure 2-7). Development time was more than 3 times longer during the 4th instar than during the 1st and 2nd instars, whereas development time was twice as long for 4th instar compared to 3rd instar. Mean larval development times were significantly different between species (t= 5.1049, df= 21, P<0.001). Minimum and maximum development times for *R. lophanthae* were 26 d and 61 d, respectively. Minimum and maximum development times for *C. montrouzieri* were 32 d and 101 d, respectively.

Later instars of *R. lophanthae* had progressively longer development times at 24°C (Figure 2-8), with total larval development time averaging 22.1 ± 6.5 d. Larvae of *C. montrouzieri* had the shortest development time (8.6 ± 2.9 d) during the 2nd instar, whereas the longest development time occurred in both 1st and 4th instars (13.6 and 13.4 d, respectively). Total larval development time averaged 47.8 ± 16.4 d in *C. montrouzieri*. There was a significant
difference between the two predators’ mean larval development times \((t= 4.4872, \text{df}= 23, P=0.0002)\). Minimum development time for \(R. lophanthae\) was 17 d and the maximum was 41 d. Larvae of \(C. montrouzieri\) developed in a minimum of 22 d and a maximum of 67 d.

Variation occurred in the development time for each instar between the two predators and the two temperatures (Figures 2-7, 2-8). Development time for all instars at both temperatures was always greater for \(C. montrouzieri\) compared to \(R. lophanthae\). At 18°C, instar development times were only significantly different between species for the 1\(^{\text{st}}\) \((t=2.2910, \text{df}=19, P=0.0336)\) and 4\(^{\text{th}}\) \((t=6.0517, \text{df}=8, P=0.0003)\) instars. There was a significant difference observed between species during the 1\(^{\text{st}}\) \((t=7.4398, \text{df}=23, P<0.0001)\), 2\(^{\text{nd}}\) \((t=4.0072, \text{df}=17, P=0.0009)\), 3\(^{\text{rd}}\) \((t=2.8646, \text{df}=15, P=0.0118)\) and 4\(^{\text{th}}\) \((t=2.8077, \text{df}=12, P=0.0158)\) instars at 24°C.

**Pupation Period**

The pupation periods for \(R. lophanthae\) and \(C. montrouzieri\) differed significantly at both temperatures (Figure 2-9). Pupation was longer for \(C. montrouzieri\) than for \(R. lophanthae\) at 18°C \((t=7.8284, \text{df}=9, P<0.001)\) and at 24°C \((t=8.2968, \text{df}=9, P<0.0001)\).

**Adult Longevity**

Adult mortality of \(R. lophanthae\) feeding only on female scales reached 50% on day 99 at 18°C and on day 94 at 24°C (Figure 2-10). Longevity data for \(C. montrouzieri\) on a diet of female scales only were not obtained because the larvae failed to complete development. Longevity data for \(C. montrouzieri\) and \(R. lophanthae\) on a diet of male and female scales were not obtained.

Female \(R. lophanthae\) longevity averaged 95.5 ± 2.9 d \((n=6)\) at 18°C, whereas longevity at 24°C averaged 104.3 ± 24.3 d \((n=9)\). Minimum and maximum longevity at 18°C were 92 d and 100 d, respectively. Minimum and maximum longevity at 24°C were 67 d and 126 d, respectively.
Male *R. lophanthae* longevity averaged 116.1 ± 19.7 (n=14) at 18°C, whereas longevity at 24°C averaged 102.6 ± 29.0 (n=12). Minimum and maximum longevity at 18°C were 74 d and 129 d, respectively. Minimum and maximum longevity at 24°C were 60 d and 128 d, respectively.

**Fecundity**

Female *R. lophanthae* began laying eggs on average 15.7 ± 4.4 d after adult emergence at 18°C and 26.1 ± 8.2 d at 24°C (Figure 2-11). Seven females kept at 18°C produced a total of 84 eggs and nine beetles kept at 24°C produced 208 eggs within 100 days. Fifty eggs were produced by one female maintained at 24°C over its lifetime. Eggs were laid individually on the top surface of female *A. yasumatsui* armor or laid both singly or in groups of 2 to 3 eggs beneath the armor of female scales that had been preyed upon. Eggs were yellow in color and eventually became transparent prior to hatching. Peak daily egg production at both temperatures was at about 55 ± 5 d (Figure 2-11). During the 100 day oviposition period, females kept at 18°C produced on average 0.2 ± 0.2 eggs per female per day. Females kept at 24°C over a 126 day period produced on average 0.2 ± 0.3 eggs per female per day. The maximum number of eggs produced by a single female in one day at 18°C was 6 on day 54. The maximum number of eggs produced by a single female in one day at 24°C was 12 on day 51.

Female *R. lophanthae* feeding on female scales at 18°C had a net reproductive rate (R₀) of 24.6, mean generation time (T) of 96.1, and intrinsic rate of increase (rₘ) of 0.01986. At 24°C, the net reproductive rate (R₀) was 69.2, the mean generation time (T) was 75.3, and the intrinsic rate of increase (rₘ) was 0.04085.

**Discussion**

*Rhyzobius lophanthae* is a better predator of *A. yasumatsui* than *C. montrouzieri*. At 18°C, egg duration of *R. lophanthae* is longer than that of *C. montrouzieri*, whereas there is no
difference at 24°C. Longer development time of the egg and 1st instars may be disadvantageous when eggs are exposed to fluctuations in temperature, humidity, precipitation and predators for longer periods of time, resulting in higher rates of mortality. Larval survivorship was significantly greater in *R. lophanthae* than in *C. montrouzieri*.

Only *R. lophanthae* was able to complete development when offered only female *A. yasumatsui*. When presented with a mixed diet of male and female *A. yasumatsui*, both predators completed larval development and had higher levels of survivorship, indicating that time was spent feeding on male scales. Similar results were recorded by Stathas (2000) when *R. lophanthae* preyed on *A. nerii*. Immature male *A. yasumatsui* were consumed by both predators on greater than 50% of days in the adult longevity study. Larval development time and pupation period were significantly greater for *C. montrouzieri* compared to *R. lophanthae* when feeding on both male and female scales, which may support the production of more generations of *R. lophanthae*.

Female *R. lophanthae* are able to produce eggs when feeding on *A. yasumatsui*, whereas *C. montrouzieri* are unable to produce eggs at 18°C or 24°C. This indicates that *C. montrouzieri* relies on other food sources to produce eggs and therefore would not be able to sustain healthy populations in areas where plants are solely infested with *A. yasumatsui* (Frank and McCoy 2007). Adult longevity and egg production by *R. lophanthae* at 18°C and 24°C were similar, indicating that this predator is capable of surviving and reproducing within that temperature range. Similar peaks in production of eggs at both temperatures indicate that fluctuations in temperature would not effect on the time frame in which the greatest numbers of eggs are produced. Further comparisons are needed to determine if egg production by *R. lophanthae* coincides with increased infestations of *A. yasumatsui* between June and September in Florida.
Figure 2-1. 20 x 9 mm Petri dish
Figure 2-2. Egg duration time of *Rhyzobius lophanthae* feeding on female *Aulacaspis yasumatsui* and *Cryptolaemus montrouzieri* feeding on *Maconellicoccus hirsutus*. Numbers inside bars indicate sample size.
Figure 2-3. Age-specific larval survivorship of *Rhyzobius lophanthae* and *Cryptolaemus montrouzieri* feeding on female *Aulacaspis yasumatsui* only.
Figure 2-4. Larval development time of *Rhyzobius lophanthae* and *Cryptolaemus montrouzieri* feeding on female *Aulacaspis yasumatsui* only at 18°C. Number in each bar represents sample size.
Figure 2-5. Larval development time of *Rhyzobius lophanthae* feeding on female *Aulacaspis yasumatsui* only at 24°C. Number in each bar represents sample size.
Figure 2-6. Age-specific larval survivorship of *Rhyzobius lophanthae* and *Cryptolaemus montrouzieri* feeding on male and female *Aulacaspis yasumatsui*.
Figure 2-7. Larval development time of *Rhyzobius lophanthe* and *Cryptolaemus montouzieri* feeding on male and female *Aulacaspis yasumatsui* at 18°C. Number in each bar represents sample size.
Figure 2-8. Larval development time of *Rhyzobius lophanthae* and *Cryptolaemus montrouzieri* feeding on male and female *Aulacaspis yasumatsui* at 24ºC. Number in each bar represents sample size.
Figure 2-9. Pupation period of *Rhyzobius lophanthae* and *Cryptolaemus montrouzieri*. Number in each bar represents sample size.
Figure 2-10. Age-specific survivorship of adult female *Rhyzobius lophanthae* feeding on female *Aulacaspis yasumatsui* only.
Figure 2-11. Mean daily egg production per female *Rhyzobius lophanthae* feeding on female *Aulacaspis yasumatsui* only.
CHAPTER 3
RHIZOBIUS LOPHANTHAE AND CRYPTOLAEMUS MONTROUZIERI CONSUMPTION RATE OF AULACASPIS YASUMATSUI

Introduction

Predaceous coccinellids have been used more often than any other predatory organisms for biological control (Obrycki 1998). The success of some scale-feeding coccinellids was hypothesized by Clausen (1940) to directly reflect the physical characteristics of the diaspid cover, the scale’s developmental stage, and the nutritional value provided (Honda and Luck 1995).

*Rhizobius lophanthae* has been an effective biological control agent against diaspидid scales in Hawaii, Italy, and Guam (Heu et al. 2003; Moore et al. 2005) through inundative releases (Stathas 2002). Cave (2006) reported isolated populations of the beetle on cycads in downtown Tampa and Tallahassee, FL. However, its limited distribution in the state could be hampering its effectiveness as a biological control agent despite the ability of *R. lophanthae* to produce as many as 8 generations per year feeding on the scale insect *Chrysomphalus dictyospermi* (Morgan) in Morroco (Smirnoff 1950). Limited distribution of coccinellid species may also reflect the suitability of the environment to sustain populations.

*Cryptolaemus montrouzieri* has proven effective in controlling mealybug and scale infestations dating back to the 1800s, having been shipped worldwide for use in biological control programs (Bartlett 1974). This species is most often used to control grape, citrus and greenhouse mealybugs. *Cryptolaemus montrouzieri*, unlike *R. lophanthae*, has been frequently encountered throughout southern Florida on *Cycas revoluta* plants infested with *Aulacaspis yasumatsui*.

*Aulacaspis yasumatsui*, originally described from Bangkok, Thailand (Takagi 1977), was first reported in 1996 infesting cycad plants in Miami, Florida. Due to the popularity of cycads in
the urban landscape, the pest quickly spread throughout southern Florida (McLaughlin 1998). Initial control of *A. yasumatsui* was attempted through the use of the insecticide, malathion and the introduction of two natural enemies, the parasitoid *Coccobius fulvus* (Compere and Annecke) and the predator *Cybocephalus nipponicus* Endrody-Younga (Howard et al. 1999; Hodges et al. 2003; Muniappan 2005).

Because of the widespread damage caused by *A. yasumatsui*, the ineffectiveness of initial control measures, and potential to spread to uninfested parts of the world, biological control agents have been sought. More information is needed to determine the level of control that could be provided by *R. lophanthae* and *C. montrouzieri*. Therefore, this study was conducted to analyze the rate of consumption of scales by *R. lophanthae* and *C. montrouzieri* throughout their development at two constant temperatures.

**Materials and Methods**

**Insects**

*Aulacaspis yasumatsui* was reared on *C. revoluta* plants kept in 3.7 L pots and maintained in a greenhouse with 30% RH. Plants were fertilized and watered regularly according to grower’s recommendations to maintain plant health. Uninfested *C. revoluta* plants were exposed to infested plants with active crawlers by interlocking leaves for 1 week, allowing crawlers to settle on the uninfested foliage. Moderately infested plants with 3rd instar *A. yasumatsui* were obtained in approximately 1 month at 30°C (Howard et al. 1999). Adult *R. lophanthae* were obtained from Rincon-Vitova Insectaries (Ventura, California) and kept in 20 × 20 × 20 cm Bug Dorms (BioQuip, Inc., Rancho Dominguez, CA) with water-saturated cotton balls and infested *C. revoluta* plants at 25°C, 60% RH and 14:10 (L:D) photoperiod. Adult *C. montrouzieri* were unable to produce eggs while feeding on *A. yasumatsui* (personal observation), therefore eggs were obtained from the Florida Department of Agriculture and Consumer Services, Division of
Plant Industry in Gainesville, FL. Eggs of *C. montrouzieri* were produced from females feeding on *M. hirsutus*.

**Experimental Design**

Ten 1\textsuperscript{st} instars and 10 adults of each sex of *R. lophanthae* and *C. montrouzieri* were placed individually in 20 × 9 mm Petri dishes with screen lids and provided daily with *C. revoluta* leaflets infested with 10-20 2\textsuperscript{nd} and 3\textsuperscript{rd} instar female *A. yasumatsui*. First instar and male scales were manually removed using a small paint brush. Beetles were kept in environmental chambers set at 18±2°C and 24±2°C, with 60% RH and 14:10 (L:D) photoperiod. Adult *R. lophanthae* used in the test were the F\textsubscript{1} progeny of adult beetles purchased from Rincon-Vitova, reared on a diet of *A. yasumatsui* whereas *C. montrouzieri* were F\textsubscript{1} progeny of beetles feeding on *M. hirsutus*. Adults were not starved prior to being studied. Both larvae and adults were scored daily until pupation or death, respectively, for number of scales consumed. Consumed scales were indicated by an absence of the female scale’s body due to complete consumption or damaged from partial feeding that resulted in a dead scale with a hole in its armor (Figure 3-1). The study was repeated five times for both temperatures.

In a second experiment, ten 1\textsuperscript{st} instars of *R. lophanthae* and *C. montrouzieri* were evaluated using the same protocol stated above. Dishes were provided daily with 10-20 2\textsuperscript{nd} and 3\textsuperscript{rd} instar female and 20-40 3\textsuperscript{rd} instar male *A. yasumatsui* on *C. revoluta* leaflets. First instars and male 2\textsuperscript{nd} instars were manually removed using a small paint brush. Larvae of both predator species were scored daily until pupation or death, respectively, for number of female scales consumed and visible damage to male scales. The study was repeated five times for both temperatures.

Mean consumption rates were compared between predator species and temperatures using an analysis of variance (ANOVA) (Proc GLM, SAS Institute, 2001) and a t-test to separate
means. Means are reported with their standard error. Data for all 5 studies were combined during the analysis.

**Results**

**Larvae**

Daily consumption varied considerably between larvae of *R. lophanthae* and *C. montrouzieri* feeding on female *A. yasumatsui* at 18°C and 24°C (Tables 3-1, 3-2). Daily and total consumption increased with development into subsequent instars for both species at 18°C. Daily and total consumption of scales was significantly greater for *R. lophanthae* during the 1st 

(t=3.0165, df=429, P=0.0027), 2nd (t=4.4164, df=161, P<0.001) and 3rd (t=5.8763, df=201, P<0.001) instars at 18°C compared to *C. montrouzieri* (Table 3-1). Daily consumption was 2 times greater for 1st instar *R. lophanthae*, 7 times greater for 2nd instar, and 5 times greater for third instar compared to daily consumption by the respective instars of *C. montrouzieri* at 18°C. Larvae of *C. montrouzieri* failed to complete the 4th instar while feeding on female scales. Total consumption of scales was significantly greater by *R. lophanthae* than *C. montrouzieri* during the 1st (t=150.4072, df=50, P<0.001), 2nd (t=9.6746, df=24, P<0.001) and 3rd instars (t=17.7223, df=21, P<0.001) at 18°C.

At 24°C, daily consumption by 1st instar *R. lophanthae* was significantly greater (t=3.2040, df=323, P=0.0015) than by *C. montrouzieri*. Larvae of *C. montrouzieri* failed to complete the 2nd instar. Total consumption by 1st instars was also greater in *R. lophanthae* (t=3.2979, df=323, P=0.0011) at this temperature; daily and total consumption were about 2 times greater for *R. lophanthae* compared to *C. montrouzieri*.

Consumption by cohorts of *R. lophanthae* and *C. montrouzieri* larvae feeding on male and female *A. yasumatsui* varied significantly at 18°C and 24°C (Tables 3-3, 3-4). Daily consumption was greater by *R. lophanthae* during the 3rd (t=7.7791, df=223, P<0.001) and 4th...
instars at 18°C compared to \textit{C. montrouzieri} (Table 3-3). Daily consumption by \textit{R. lophanthae} was 27 times greater for 3\textsuperscript{rd} instars and 3 times greater for 4\textsuperscript{th} instars compared to 3\textsuperscript{rd} and 4\textsuperscript{th} instars of \textit{C. montrouzieri}. Total consumption by each instar at 18°C again revealed higher levels of consumption of \textit{A. yasumatsui} by \textit{R. lophanthae} during the 2\textsuperscript{nd} \((t=2.1202, \, df=16, \, P=0.0500)\), 3\textsuperscript{rd} \((t=26.3366, \, df=15, \, P<0.001)\), and 4\textsuperscript{th} \((t=4.8122, \, df=7, \, P=0.0019)\) instars compared to \textit{C. montrouzieri}. The average total consumption of scales during the entire larval development reflected a similar trend \((t=18.4397, \, df=65, \, P<0.001)\). Total consumption by \textit{R. lophanthae} was 9 times greater for 3\textsuperscript{rd} instars, 2 times greater for 4\textsuperscript{th} instars, and 3 times greater for the entire larval period compared to \textit{C. montrouzieri}.

At 24°C, cohorts of \textit{R. lophanthae} feeding on male and female \textit{A. yasumatsui} consumed significantly greater numbers of scales daily during the 3\textsuperscript{rd} \((t=1.9731, \, df=187, \, P<0.001)\) and 4\textsuperscript{th} \((t=3.3381, \, df=166, \, P=0.0010)\) instars than \textit{C. montrouzieri} (Table 3-4). Daily consumption was 2 times greater in 4\textsuperscript{th} instar \textit{R. lophanthae} compared to 4\textsuperscript{th} instar \textit{C. montrouzieri} at 24°C. Total consumption of scales was greater by \textit{C. montrouzieri} during the 1\textsuperscript{st} \((t=8.6049, \, df=23, \, P<0.001)\) and 2\textsuperscript{nd} \((t=7.9293, \, df=18, \, P<0.001)\) instars compared to total consumption by \textit{R. lophanthae} at 24°C; there was no significant difference between species for the other instars nor total larval period (Table 3-4).

**Adults**

Adult female \textit{R. lophanthae} consumed greater numbers of female scales daily than male beetles at 18°C \((t=3.1391, \, df=424, \, P=0.0018)\) (Table 3-1). The total average consumption was also significantly greater for female \textit{R. lophanthae} \((t=8.2328, \, df=18, \, P<0.001)\). At 24°C, adult female \textit{R. lophanthae} consumed significantly greater numbers of female \textit{A. yasumatsui} daily \((t=6.9095, \, df=1695, \, P<0.001)\) than males. Average total consumption by female \textit{R. lophanthae} compared to males was significantly higher \((t=9.1913, \, df=19, \, P<0.001)\).
Discussion

When offered a diet of only female *A. yasumatsui*, *R. lophanthae* larvae were able to complete development at 18°C and 24°C, unlike *C. montrouzieri*. This suggests that female scales may not provide proper nutrition for development by the latter species. The lesser consumption of female scales by *C. montrouzieri* may also reflect mechanical difficulty of larvae to penetrate the armor of female *A. yasumatsui*. Structural differences in the mandibles of generalist coccinellids versus that of species specialized for feeding on armored scales may affect the rate of consumption and development of predators. In a study by Honda and Luck (1995), the mandibular structures of adult *R. lophanthae* and *Chilocorus cacti* (L.) were compared when feeding on hard and soft scales. Differences in the structures of each species’ mandibles were better adapted to removing scale covers of some species over others and therefore had an effect on a beetle’s selection of a host.

Female *R. lophanthae* consumed approximately two-thirds more scales than males, as was similarly observed by Stathas (2000). Research on longevity of beetles under these conditions did not show significant variation in life span, therefore this difference in consumption may indicate the need for more resources to begin oviposition. Higher numbers of *A. yasumatsui* were consumed at 24°C than at 18°C which may be due to a shorter development time at the higher temperature and the need for more nutrition in a shorter period of time to complete development.

Both species were able to complete development when presented a diet of male and female *A. yasumatsui*. In this study, *C. montrouzieri* larvae were observed feeding almost exclusively on male *A. yasumatsui* and selectively avoiding female scales. Male scales, being more numerous and having thinner coverings, may require less energy to consume. Individuals
of *R. lophanthae* also consumed significantly fewer female scales when offered both male and female *A. yasumatsui*.

This study revealed the rates of consumption by *R. lophanthae* were higher than that of *C. montrouzieri* as well as the ineffectiveness of both beetles in targeting female *A. yasumatsui*, implicating their inability to control the increase of scale populations. Further investigation into biological control agents that can more precisely control fluctuations in populations of *A. yasumatsui* by targeting reproductively viable females is greatly needed.
Figure 3-1. Feeding damage to *Aulacaspis yasumatsui* by *Rhyzobius lophanthae* larva.
Table 3-1. Daily and total consumption of 2\textsuperscript{nd} and 3\textsuperscript{rd} instar female *Aulacaspis yasumatsui* by *Rhyzobius lophanthae* and *Cryptolaemus montrouzieri* larvae and adults at 18\degree C. N is the number of days on which the average daily consumption rate is based; n is the number of individuals on which the average total consumption rate is based. Means and standard errors are compared across columns to compare the same instar in both species. Means with the same letter are not significantly different (p>0.05).

<table>
<thead>
<tr>
<th>18\degree C</th>
<th><em>Rhyzobius lophanthae</em></th>
<th><em>Cryptolaemus montrouzieri</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Daily Consumption</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>Mean±SE</td>
</tr>
<tr>
<td>1st</td>
<td>232</td>
<td>0.3±0.5</td>
</tr>
<tr>
<td>2nd</td>
<td>138</td>
<td>1.2±1.1</td>
</tr>
<tr>
<td>3rd</td>
<td>182</td>
<td>2.7±1.7</td>
</tr>
<tr>
<td>4th</td>
<td>120</td>
<td>2.4±2.3</td>
</tr>
<tr>
<td>Adult Males</td>
<td>295</td>
<td>2.3±1.3</td>
</tr>
<tr>
<td>Adult Females</td>
<td>131</td>
<td>2.7±1.4</td>
</tr>
<tr>
<td></td>
<td>Total Consumption</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Mean±SE</td>
</tr>
<tr>
<td>1st</td>
<td>20</td>
<td>2.7±0.4</td>
</tr>
<tr>
<td>2nd</td>
<td>20</td>
<td>8.1±0.9</td>
</tr>
<tr>
<td>3rd</td>
<td>20</td>
<td>25.6±1.9</td>
</tr>
<tr>
<td>4th</td>
<td>20</td>
<td>39.0±2.4</td>
</tr>
<tr>
<td>Larvae</td>
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<td>75.4±5.6</td>
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<td>Adult Males</td>
<td>14</td>
<td>121.9±13.5</td>
</tr>
<tr>
<td>Adult Females</td>
<td>6</td>
<td>175.8±13.2</td>
</tr>
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Table 3-2. Daily and total consumption of 2\textsuperscript{nd} and 3\textsuperscript{rd} instar female *Aulacaspis yasumatsui* by *Rhyzobius lophanthae* and *Cryptolaemus montrouzieri* larvae and adults at 24°C. N is the number of days on which the average daily consumption rate is based; n is the number of individuals on which the average total consumption rate is based. Means and standard errors are compared across columns to compare the same instar in both species. Means with the same letter are not significantly different (p>0.05).

<table>
<thead>
<tr>
<th>Instar</th>
<th>24°C</th>
<th>Rhyzobius lophanthae</th>
<th>N</th>
<th>Mean±SE</th>
<th>Cryptolaemus montrouzieri</th>
<th>N</th>
<th>Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Daily Consumption</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>112</td>
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<td>213</td>
<td>0.1±0.3 b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd</td>
<td>109</td>
<td>2.0±2.0</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd</td>
<td>76</td>
<td>4.8±2.6</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4th</td>
<td>118</td>
<td>4.9±4.4</td>
<td>-</td>
<td>-</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adult Males</td>
<td>876</td>
<td>2.6±1.5</td>
<td>-</td>
<td>-</td>
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<td>Adult Females</td>
<td>821</td>
<td>3.1±1.6</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total Consumption</td>
<td>n</td>
<td>Mean±SE</td>
<td>N</td>
<td>Mean±SE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>21</td>
<td>1.1±0.3 a</td>
<td>36</td>
<td>0.5±2.7 a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd</td>
<td>21</td>
<td>10.0±1.0</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd</td>
<td>21</td>
<td>17.9±1.6</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4th</td>
<td>21</td>
<td>29.4±2.9</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Larvae</td>
<td>21</td>
<td>58.4±5.8</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adult Males</td>
<td>12</td>
<td>194.0±22.6</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td>Adult Females</td>
<td>9</td>
<td>281.0±19.8</td>
<td>-</td>
<td>-</td>
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</tr>
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</table>
Table 3-3. Daily and total consumption of 2nd and 3rd instar male and female *Aulacaspis yasumatsui* by *Rhyzobius lophanthae* and *Cryptolaemus montrouzieri* larvae at 18°C. N is the number of days on which the average daily consumption rate is based; n is the number of individuals on which the average total consumption rate is based. Means and standard errors are compared across columns to compare the same instar in both species. Means with the same letter are not significantly different (p>0.05).

<table>
<thead>
<tr>
<th>18°C</th>
<th>Rhyzobius lophanthae</th>
<th>Cryptolaemus montrouzieri</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Daily Consumption</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N           Mean±SE</td>
<td>N           Mean±SE</td>
</tr>
<tr>
<td>1st</td>
<td>40          0.1±0.3 a</td>
<td>216             &lt;0.1±0.2 a</td>
</tr>
<tr>
<td>2nd</td>
<td>31          0.1±0.3 a</td>
<td>140             &lt;0.1±0.2 a</td>
</tr>
<tr>
<td>3rd</td>
<td>12          0.8±1.2 a</td>
<td>213             &lt;0.1±0.2 b</td>
</tr>
<tr>
<td>4th</td>
<td>58          0.7±1.2 a</td>
<td>339             0.2±0.8 b</td>
</tr>
<tr>
<td></td>
<td>Total Consumption</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n           Mean±SE</td>
<td>n           Mean±SE</td>
</tr>
<tr>
<td>1st</td>
<td>6           0.3±0.2 a</td>
<td>17             0.2±0.1 a</td>
</tr>
<tr>
<td>2nd</td>
<td>5           0.8±0.5 a</td>
<td>13             0.5±0.1 b</td>
</tr>
<tr>
<td>3rd</td>
<td>5           7.5±0.8 a</td>
<td>12             0.8±0.3 b</td>
</tr>
<tr>
<td>4th</td>
<td>4           15.4±4.7 a</td>
<td>5              5.5±0.3 b</td>
</tr>
<tr>
<td>Larvae</td>
<td>4         24.0±6.1 a</td>
<td>5              7.3±0.9 b</td>
</tr>
</tbody>
</table>
Table 3-4. Daily and total consumption of 2\textsuperscript{nd} and 3\textsuperscript{rd} instar male and female *Aulacaspis yasumatsui* by *Rhyzobius lophanthae* and *Cryptolaemus montrouzieri* larvae at 24\textdegree C. N is the number of days on which the average daily consumption rate is based; n is the number of individuals on which the average total consumption rate is based. Means and standard errors are compared across columns to compare the same instar in both species. Means with the same letter are not significantly different (p>0.05).

<table>
<thead>
<tr>
<th>24\textdegree C</th>
<th><em>Rhyzobius lophanthae</em></th>
<th><em>Cryptolaemus montrouzieri</em></th>
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<tbody>
<tr>
<td></td>
<td>Daily Consumption</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>Mean±SE</td>
</tr>
<tr>
<td>1st</td>
<td>38</td>
<td>0.3±0.6 a</td>
</tr>
<tr>
<td>2nd</td>
<td>36</td>
<td>0.7±1.0 a</td>
</tr>
<tr>
<td>3rd</td>
<td>45</td>
<td>1.5±1.4 a</td>
</tr>
<tr>
<td>4th</td>
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</tr>
<tr>
<td></td>
<td>Total Consumption</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Mean±SE</td>
</tr>
<tr>
<td>1st</td>
<td>9</td>
<td>0.3±0.2 a</td>
</tr>
<tr>
<td>2nd</td>
<td>6</td>
<td>0.8±0.5 a</td>
</tr>
<tr>
<td>3rd</td>
<td>6</td>
<td>7.5±0.8 a</td>
</tr>
<tr>
<td>4th</td>
<td>6</td>
<td>15.4±4.7 a</td>
</tr>
<tr>
<td>Larvae</td>
<td>6</td>
<td>24.9±6.1 a</td>
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</table>
CHAPTER 4
FIELD RELEASE STUDY OF RHYZOBIUS LOPHANTHAE

Introduction

*Rhizobius lophanthae* is an important natural enemy of many armored scale species and has been introduced into Hawaii, Italy, and Guam for control of scales (Yus 1973; Rosen 1990; Heu et al. 2003; Moore et al. 2005). This species is an effective biological control agent due to biological factors such as its prey specificity, high fecundity and adult longevity as well as ecological factors including its lack of diapause, high mobility, lack of parasitism and rapid population development (Statthas et al. 2002). Beetles are able to feed on armored scales by straddling the scale while maintaining contact with the substrate and inserting their mandibles under the scale cover while pressing away from the substrate. Studies with *R. lophanthae* demonstrated the predators’ ability to handle smaller scales, especially the immature stages (Honda 1999).

*Aulacaspis yasumatsui* Takagi is an invasive armored scale native to a region stretching from the Andaman Islands to Vietnam, Thailand, southern China, and likely Cambodia, Laos, Malaysia, and Myanmar (Howard et al. 1999; Muniappan 2005). Since its accidental introduction into the US in 1996, *A. yasumatsui* has spread to more than 7 southern states (Broome 2000). This species has also been detrimental to plants in the West Indies, Guam, Hong Kong, Singapore, Taiwan, New Zealand, Costa Rica, and Africa (Weissling et al. 1999; Hodges et al. 2004; Moore et al. 2005; Germain and Hodges 2007).

The scale gives plants an unsightly snow-covered appearance that when unmanaged can form dense multilayered coverings of nearly 3,000 scales per square inch (Weissling et al. 1999), eventually leading to the death of plants (Heu et al. 2003). Management of the scale infestations is hindered by its ability to infest the primary and secondary roots of plants as deep as 60 cm into
the ground (Howard et al. 1999). Additionally, scales can escape freezing temperatures by hiding in overlapping plant material from previous years’ growth in the central cone of *Cycas revoluta* plants, which cannot be reached with normal chemical treatments (Broome 2002).

While *R. lophanthae* is commercially available as a biological control agent, information regarding the adequate number of *R. lophanthae* needed to control an infestation of *A. yasumatsui* per plant is unknown. This study was undertaken to assess the number of beetles needed to control an infestation of *A. yasumatsui* on a given leaf area over a period of time in a greenhouse and urban environment. The amount of consumption by *R. lophanthae* on plants will provide a standard for treating infested plants.

**Materials and Methods**

**Greenhouse Study**

**Insects**

*Aulacaspis yasumatsui* was reared on *C. revoluta* plants kept in 3.7 L pots and maintained in a greenhouse with 30% RH. Plants were fertilized and watered regularly according to grower’s recommendations to maintain the health of plants throughout the study. Clean plants were exposed to infested plants with active crawlers by interlocking leaves for 1 week, allowing crawlers to settle on the clean foliage. Moderately infested plants with 3rd instar *A. yasumatsui* were obtained in approximately 1 month at 30ºC (Howard et al. 1999). Adult *R. lophanthae* were obtained from Rincon-Vitova Insectaries (Ventura, California) and kept in 20 × 20 × 20 cm Bug Dorms (BioQuip, Inc. Rancho Dominguez, CA) with water-saturated cotton balls and infested *C. revoluta* plants at 25ºC, 60% RH and 14:10 (L:D) photoperiod.
Experimental design

*Cycas revoluta* plants infested with 10-50 2nd and 3rd instar male and 5-30 2nd and 3rd instar female *A. yasumatsui* per leaflet were chosen. The initial infestation per leaf was determined by sampling 6 leaflets at random and counting the number of 2nd and 3rd instar female *A. yasumatsui*. The number of leaflets per leaf was also counted. Individual leaves were enclosed by a cylindrical mesh bag that was secured with rubber bands at the leaf base and at the tip just past the last leaflet. Mesh bags allowed conditions on the leaf to remain similar to those of the greenhouse at 29±5°C with 21% RH recorded with a HOBO® Data Logger. Bags were large enough to allow movement along upper and lower sides of leaves. Velcro closure along the underside of the leaf allowed for access to bags without removing them from the leaf.

Beetles were starved for 48 h prior to study. Leaves were randomly assigned treatments of 0, 2, 4, 6, or 8 adult *R. lophanthae* with a sex ratio of 1:1. Beetles were manually shaken into each bag. Six leaflets were sampled at random every 3 days from each leaf. The number of consumed scales and live, undamaged scales in the sample and number of live beetles in the cage were recorded. Dead *R. lophanthae* were replaced with new beetles to maintain treatment numbers for 2 weeks. Each treatment was replicated 4-5 times.

The average number of female *A. yasumatsui* consumed versus the number of scales alive and undamaged by *R. lophanthae* was analyzed using a t-test.

Field Study

Insects and plants

Adult *R. lophanthae* were obtained from Rincon-Vitova Insectaries (Ventura, California) and kept in vials with sugar water-saturated cotton balls and leaflets of infested *C. revoluta* plants at 25°C, 60% RH and 14:10 (L:D) photoperiod. Commercially produced *C. revoluta* plants in 9 L containers and mature 1m tall in ground plants used in this study were naturally infested.
prior to being selected. All plants were maintained at each site for 6 months prior to this study without the use of chemical control. Plants chosen had 10-50% of their leaf area covered in scales, were healthy and had ≥5 leaves at the beginning of the study.

**Experimental design**

Selected plants were found at one of three sites with 12 containerized plants at Site 1, 4 in ground plants at Site 2 and 12 in ground plants at Site 3. Site 1 (Figure 4-1) and Site 2 (Figure 4-2) were located less than one mile from one another in a suburban area. Both sites represented the typical landscape for homeowners. Plants at both sites were separated by at least 2 m from one another. Site 3 (Figure 4-3) was situated in a high foot-traffic area in downtown Tampa that uses cycads in a business landscape. Plants were sampled prior to releasing predators. At Site 1 and Site 2 parasitism by *Coccobius fulvus* (Compere and Annecke) was observed with a maximum of 6 parasitized scales per sample of 6 leaflets and a maximum of 21 parasitized scales at Site 3. The beetle *Cybocephalus nipponicus* Endrödy-Younga on a single day was also observed on all plants at Site 2 with a maximum of 36 beetles and minimum of 3 beetles on one plant. Infested plants at each site were randomly assigned 0, 100, 200 or 300 adult *R. lophanthae* with a 50:50 sex ratio. Just after sunset at 6pm, beetles were transferred from plastic vials onto plants where the leaves met the trunk by manually shaking the vial (Figure 4-4). Plants were sampled every 4 d from September until November during a peak in *A. yasumatsui* infestation. Each plant was scored in the field for level of scale infestation as percentage of leaf area covered (low 10-40%, medium 41-70%, and heavy 71-100%), number of *R. lophanthae* larvae and adults observed, presence of other scale predators and beetle predation damage to scales. Consumption rates were determined by sampling 6 leaflets randomly from each plant every 8 d and by counting the number of live scales present on each leaflet, occurrence of feeding damage by beetles and parasitoid emergence holes observed using a stereomicroscope.
Results

Greenhouse Study

The greatest mean number of scales consumed per beetle occurred in the treatment of 8 beetles per leaf (Figure 4-5) with a decreasing number of scales consumed as the number beetles in each treatment decreased. High rates of beetle mortality were observed in all treatments. In order to determine the consumption of beetles prior to death, assuming they did not consume the maximum number of scales possible yet they lived long enough to consume some scale, all dead beetles were counted as half of one individual when calculating the average number of scales consumed. Similar trends in consumption were observed in all treatments demonstrating an increase in consumption over time.

Treatments with higher numbers of beetles consumed a greater proportion of the total scale population (Figure 4-6). The proportion of scales consumed by treatments of 8 beetles was significantly greater than those with 2 beetles over the entire study and greater than treatments with 4 beetles in the first 9 d. Six beetles consumed significantly more scales than 2 beetles during the whole period except 9 d after release. Based on regression lines for each treatment, 50% mortality of scales on one leaf is possible in 12, 16, 79 and 525 d, respectively, for treatments of 8, 6, 4 and 2 beetles.

Healthy scale infestations were reduced by 10% following the introduction of *R. lophanthae* in treatments with 4 and 6 beetles whereas treatments with 2 beetles were reduced by 35% (Figure 4-7).

Field Study

Adult *R. lophanthae* were observed on plants during the first 12 d at Site 1, first 4 d at Site 2, and first 8 d at Site 3 following their release (Figure 4-4). After 4 d, plants with 100 beetles released at Site 1 had an average of 5 beetles per plant, with a minimum of 0 and
maximum of 14. Plants with 200 beetles released had 11 beetles on average per plant, with a minimum of 2 and maximum of 21. Plants with 300 beetles released had on average 17 beetles per plant, with a minimum of 14 and maximum of 19. After 8 d, all plants at Site 1 had \( \leq 6 \) beetles per plant. At Site 2, 5 beetles were observed 4 d after the release of 100 beetles and 6 beetles were observed on plants after 300 beetles were released. Plants at Site 3 with 100 beetles released had an average of 1 beetle per plant 4 d after release. Plants treated with 200 beetles had an average of 3 beetles per plant, with a minimum of 0 and maximum of 6, while plants treated with 300 beetles had an average of 2 beetles per plant, with a minimum of 0 and maximum of 3. After 8 days at Site 3, 1 beetle was observed on each of two plants treated with 200 and 300 beetles. No beetles were found on subsequent days at Sites 2 or 3.

Larvae of \( R. \ lophanthae \) were observed 20-28 d following release of adults at Site 1 and 12-24 d at Site 3 (Table 4-1). No larvae were observed at Site 2. Five larvae were seen on day 20 at Site 1 with a decline in the number of larvae observed on following days, with 2 larvae at 24 d and 1 larva at 28 d. At Site 3, 1 larva was observed on day 12 and again on day 24. There was no correlation between the number of beetles released and the number of larvae observed.

Initial beetle feeding damage was observed on infested plants during the first 8 d. With the absence of beetles and low numbers of larvae, there were no significant differences in damage to scales among treatments at later time points. The level of scale infestation increased over the course of the study following release of \( R. \ lophanthae \) (Figure 4-8). At Site 1, all control plants had a heavy infestation 20 d after the initial release. All plants at this site had a medium to heavy infestation by day 36, regardless of treatment. Plants at Site 2 maintained very low to medium infestations during the entire study. At Site 3, control plants were heavily infested after 36 d. All other plants at Site 3 had medium to heavy infestations after 24 d. Levels of \( A. \)
*yasumatsui* infestation on 4 plants were so high that 44 d after release, the plants were removed due to poor health and to decrease the further spread of scales.

Several species of predatory beetles and evidence of parasitism were observed on the plants. Adults of *C. nipponicus* were found at all three sites, with the largest population at Site 2. As many as 36 beetles were observed on one plant at Site 2. The coccinellids *Chilocorus stigma* (Walker) and *Exochomus childreni* Mulsant were both observed at Sites 2 and 3, while *Curinus coeruleus* Mulsant adults were present only at Site 3. As many as 4 *C. stigma*, 7 *E. childreni* and 1 *C. coeruleus* were observed on a single plant at one time. The presence of *C. fulvus* at Sites 1 and 2 was also observed.

**Discussion**

In the greenhouse study, 8 beetles consumed more scales than treatments with 2, 4 or 6 beetles. These results may suggest that with a larger number of beetles, individuals were better able to search leaf areas for undamaged scales to feed on. The average number of scales consumed by 8 beetles was significantly greater than that of 2 beetles. There was no significant difference between all other treatments. Analysis of the proportion of total scales consumed indicated that increased numbers of beetles are able to control populations of *A. yasumatsui* in the shortest amount of time. These results follow with the previous results indicating that the application of more beetles to a plant may result in the control of scales below an economic injury level in a shorter period of time. Beetle mortality rates were high, with as many as 5 beetles in a treatment found dead. The high humidity in the greenhouse and restricted movement of beetles to any other part of the plant may have contributed to their mortality. Scale populations initially decreased after the release of *R. lophanthae* in all treatments and then held relatively constant for all treatments except those with 2 beetles. These results were unexpected. The restricted search area should have increased the likelihood of adults finding healthy scales.
and should have resulted in higher levels of control than those observed. Because beetles were starved prior to their release the data may reflect initial heavy feeding damage.

In the field study, the high mobility of *R. lophanthae* after release may have contributed to its dispersal away from treatment plants despite the availability of scales on the plants. Interference competition as a result of large numbers of beetles in each treatment may also have influenced this result. The presence of large numbers of *C. nipponicus* at Site 2 may have been effective in maintaining scale infestations at low levels. All other beetle species were observed on plants in which scale populations were increasing, therefore populations of those species were apparently not effective in controlling *A. yasumatsui*. Both Site 2 and Site 3 were located along roadways which may have made the sites less ideal for populations of beetles to establish with the movement of air as vehicles passed by.

Given that the infestation of *A. yasumatsui* was higher on all plants at the end of the field study and the high mortality of beetles and low level of control of scales in the greenhouse, I would not recommend using *R. lophanthae* as a biological control agent following the same parameters of this experiment. Similar releases have taken place at field sites in Guam, however, in those releases beetles were kept in mesh sleeves on plants for several weeks before bags were removed (Moore et al. 2005). This may have allowed beetles to spend more time laying eggs on the plant before dispersing.
Figure 4-1. Field release Site 1. Blue represents water, brown is a residence, black is a paved surface, green area is grass, and dark circles represent individual plants used and their treatment (replicate-number of beetles released).
Figure 4-2. Field release Site 2. Black objects are paved surfaces, green represents grass, and dark green circles indicate individual plants used and their treatment (replicate-number of beetles released).
Figure 4-3. Field release Site 3. Black indicates a paved surface, brown indicates a building, green represents grass, and dark green circles represent individual plants used and their treatment (replicate-number of beetles released).
Figure 4-4. Adult *R. lophanthae* were manually shaken onto a *C. revoluta* plant infested with *A. yasumatsui.*
Figure 4-5. Average number of 2\textsuperscript{nd} and 3\textsuperscript{rd} instar female \textit{A. yasumatsui} consumed per \textit{R. lophanthae} adult in treatments of 0, 2, 4, 6, and 8 beetles per leaf. Data account for mortality of adult beetles in a greenhouse.
Figure 4-6. Proportion of total *A. yasumatsui* population consumed by *R. lophanthae* in treatments of 0, 2, 4, 6 and 8 beetles per leaf in a greenhouse.
Figure 4-7. The mean number of healthy \textit{A. yasumatsui} per leaflet, undamaged by \textit{R. lophanthae} over time in a greenhouse.
Table 4-1. Number of *Rhzobius lophanthae* observed on cycad plants at field sites after their release.

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Figure 4-8. The percentage of leaf area infested by *A. yasumatsui* over time in treatments of 0, 100, 200 or 300 *R. lophanthae* over all sites in the field.
LIST OF REFERENCES


ONSET COMPUTER CORPORATION (1996-2007) Bourne, MA, USA


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BIOGRAPHICAL SKETCH

Greta Thorson received her Bachelor of Science degree from the University of Delaware in 2006, majoring in entomology with a minor in landscape horticulture. From 2002 to 2004, she worked as a biological science technician at the USDA Beneficial Insects Research Laboratory in Newark, DE under the direction of Mr. Roger Fuester. While working for the USDA she assisted with research on the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) and gypsy moth parasitoids. During the summers of 2003 and 2004 she worked at the USDA Bee Research Lab in Beltsville, MD for Mr. I. Barton Smith as a biological science technician tending to honey bee colonies and assisting with research. She worked in the Termite Research and Genetics lab of Dr. Susan Whitney King at the University of Delaware from May 2005 until July 2006, focusing on colony behavior and variation between *Reticulitermes flavipes* (Kollar) and *Reticulitermes virginicus* (Banks). Most recently she worked as a graduate research assistant with the University of Florida from 2006-2008.